



The mechanism of lung tissue YKL-40 promoting the interstitial transformation of alveolar epithelial cells and its effect on TGF- β 1 level in mice with idiopathic pulmonary fibrosis

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ABSTRACT

This study aimed to investigate the mechanism of lung tissue YKL-40 promoting the interstitial transformation of alveolar epithelial cells in mice with idiopathic pulmonary fibrosis and its effect on the level of TGF- β 1. For this purpose, Forty SPF SD mice were randomly divided into 4 groups. They were the blank control group (CK group), virus-negative control group (YKL-40-NC group), YKL-40 knockdown group (YKL-40-inhibitor group) and YKL-40 overexpression group (YKL-40-mimics group), respectively. The mRNA expressions of alveolar epithelial cell mesenchymal transformation-related proteins, pulmonary fibrosis-related factors and TGF- β 1-related pathway proteins in the above four groups of mice were compared to determine the mechanism of the promotion of alveolar epithelial cell mesenchymal transformation by YKL-40 in the lung tissues of mice with idiopathic pulmonary fibrosis and the effect of YKL-40 on the level of TGF- β 1. The results showed that in terms of lung wet/dry weight ratio, the YKL-40-NC group, YKL-40-inhibitor group and YKL-40-mimics group were significantly increased compared with the CK group ($P < 0.05$). About YKL-40 protein expression, compared with the CK group, AOD value and YKL-40 protein expression in the YKL-40-NC group, YKL-40-inhibitor group and YKL-40-mimics group were significantly increased ($P < 0.05$), and compared with YKL-40-NC group, The AOD value and YKL-40 protein expression in YKL-40-inhibitor group were significantly decreased, while the AOD value and YKL-40 protein expression in YKL-40-mimics group were significantly increased ($P < 0.05$), suggesting successful lentivirus transfection. Compared with the CK group, β -catenin and E-cadherin in the alveolar epithelial cells were significantly increased, while Pro-SPC was significantly decreased ($P < 0.05$). The mRNA expression of pulmonary fibrosis-related factors showed that compared with the CK group, the mRNA expression of viminin and hydroxyproline was significantly increased, while the mRNA expression of E-cadherin was decreased ($P < 0.05$). However, the mRNA expressions of viminin and hydroxyproline in the YKL-40-inhibitor group were significantly decreased, but the mRNA expression of E-cadherin was significantly increased. Compared with CK group, the protein expressions of TGF- β 1, Smad3, Smad7 and α -Sma in the CK group were significantly increased ($P < 0.05$). The protein expressions of TGF- β 1, Smad3, Smad7 and α -SMA in the YKL-40-mimics group were significantly increased, but the protein expressions of TGF- β 1, Smad3, Smad7 and α -SMA in YKL-40-inhibitor group were significantly decreased ($P < 0.05$). In general, overexpression of YKL-40 can promote the progression of pulmonary fibrosis and the interstitial transformation of alveolar epithelial cells in mice with idiopathic fibrosis.

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Introduction

Idiopathic pulmonary fibrosis is a diffuse interstitial lung disease of a kind of type, more common in idiopathic interstitial pneumonia, early pathological characteristics are mainly characterized by diffuse alveolar inflammation, alveolar structure disorder late is characterized by abnormal hyperplasia of fibroblasts and extracellular matrix accumulation and collagen content increases, eventually, lead to pulmonary fibrosis (1). The main clinical manifestations of this disease are respiratory disturbance accompanied by irritant dry cough, and there is usually a burst

sound at the end of inspiration in the middle and lower part of both lungs (2, 3). Due to the insidious onset of this disease, patients with symptoms usually have a disease history of 1-3 years, and most of them have the symptoms of decreased lung function (4). In recent years, due to increasing air pollution and changes in life and population structure, the incidence of the disease has been increasing year by year all over the world. Strongman et al. (5) made statistics on the morbidity and mortality of patients in the UK, and the results showed that 4527 cases of idiopathic fibrosis were found in 9748108 primary care patients, and the incidence increased by nearly 80% compared with

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2000 by 2012, and the median survival was 3 years. The above research results all indicate the high morbidity and mortality of IPF, but the pathogenesis of IPF is not clear at present, so it is of great significance to explore its pathogenesis. However, the YKL-40 protein is expressed in the lung, liver, kidney and other tissues, and is involved in the pathophysiological process of pulmonary fibrosis, sarcoidosis, asthma and other diseases. For example, Tong et al. (6) showed that the level of YKL-40 in bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis was significantly higher than that of normal people. The Transforming growth factor β 1 (TGF- β 1) is also a conduction factor in the classic TGF- β 1/Smads signaling pathway in the pathogenesis of fibrous diseases (7-9). At present, there are few studies on the correlation between idiopathic fibrosis and YKL-60 and TGF- β 1 at home and abroad. Therefore, mice with idiopathic fibrosis were selected as the research object in this study to explore the mechanism of YKL-40 promoting interstitial transformation of alveolar epithelial cells and its effect on TGF- β 1, so as to provide reference for clinical diagnosis and treatment of idiopathic fibrosis. The results are as follows.

Materials and Methods

Experimental animals

In this study, 40 SPF female SD mice were selected as the research objects. They were raised in the SPF breeding room of Nanjing Experimental Animal Center, and all animal experiments met the requirements of the Ethics Committee.

Materials and instruments

The used materials and instruments were lung administration of mice with high-pressure spray injection (MSA-250-M, Penn-Centurt), Fluorescence inverted phase contrast microscope (Beijing Borris Technology Co., LTD.), HE staining and immunohistochemical routine staining reagent (Feijing Scientific Research); Bleomycin (pritaceae), Sodium pentobarbital (Beijing); Protein imprinting luminescence system (THERmerfeld), Fluorescence quantitative PCR kit (Bosco Bio); Iodophor disinfectant (Guangdong Qilu Pharmaceutical), Overexpression of yKL-40 lentivirus plasmid and knockdown of YKL-40 lentivirus plasmid (constructed and packaged by Kolai Bio), and Surgical Instruments (Shanghai Medical Instruments Co., LTD.).

Model construction and animal grouping

In this study, 40 SD mice were selected as research objects and divided into two groups: blank control group ($n = 10$) and model group ($n = 30$). In the model group, SD mice were given 2% pentobarbital sodium 30mg/kg intraperitoneal injection and gradually exposed to the trachea. Then, bleomycin 5mg/kg was injected into the trachea at a time. After injection, the rats were rotated upright so that the drug solution was evenly distributed in the lung. Then, the wound was sutured and modeling was completed. The blank control group received the same amount of normal saline as bleomycin in the model group. Then, the model group was transfected with lentivirus, and the virus-negative control group, yKL-40 overexpression group and YKL-40 knockdown group were established (10). 293FT cells infected with 5ul lentivirus were absorbed by using a

high-pressure spray syringe for pulmonary administration in the case of the exposed trachea of mice in the model group. The syringe needle was slowly inserted into the throat along the left side of the mouth corner of the mouse. After confirming that the needle entered the trachea under direct vision, the lentivirus was immediately pushed into the airway of the mouse, and then the syringe was quickly pulled out. Under the condition of stable respiratory status of mice, surgical incisions were quickly sutured, iodophor was disinfected, and mice were continued to be fed after resuscitation.

Therefore, the blank control group (CK group), virus-negative control group (YKL-40-NC group), YKL-40 knockdown group (YKL-40-inhibitor group) and yKL-40 overexpression group (YKL-40-MIMics group) were established in this study, and all of them were sacrificed after feeding on the 10th day. The mRNA expressions of interstitial transformation-related proteins, pulmonary fibrosis-related factors and TGF- β 1 related pathway proteins in alveolar epithelial cells of mice in four groups were determined.

Histopathology and immunohistochemistry

The lung tissues of the four groups of mice were fixed and embedded with neutral formalin after death. Paraffin tissue sections were routinely prepared and stained with HE (11): The sections were placed in xylene solution for 5-10min dewaxing and were placed the dewaxed slices in a mixture of pure alcohol = 1:1 in a two-stage pump for 5min. Then gradient dehydration was carried out in 100%, 95%, 85% and 70% alcohol respectively, and the gradient placement time was about 2~5min, and then the dyeing solution was injected with distilled water and placed in hematoxylin staining solution for 5~15min. They washed the excess staining solution on the slide with water, and then they were separated under the microscope with 0.5-1% hydrochloric acid alcohol until the periphery became bright, about a few 10s. After the above was completed, it was placed into the saturated solution of lithium carbonate for alkalization, so that its nucleus became blue, and then it was washed in distilled water for a short time. It was stained with 0.1~0.5% eosin solution for 1~5min. Gradient alcohol dehydration was carried out after dyeing, each gradient was about 2~3min, then xylene transparent twice, about 10min. Finally, the slice was sealed, wiped off the excess xylene was near the slice, then quickly an appropriate amount of neutral gum was added, and then sealed the slice with a covered glass.

In this study, the conventional SP method was used for immunohistochemistry. The lung histopathological sections were placed in xylene solution for 5-10 min dewaxing. Then they have placed the dewaxed slices in a mixture of pure alcohol = 1:1 in a two-stage pump for 5min. Gradient dehydration was carried in 100%, 95%, 85% and 70% alcohol, respectively. The gradient placement time was about 2~5min. Then they were rinsed with PBS solution for 5min, 3 times. The endogenous peroxidase activity was eliminated by incubation with 3% H_2O_2 solution for 10min at room temperature. Citrate buffer was used for antigen repair, and the water bath was heated for 45min and then cooled for 45min. Goat serum was added and it was incubated for 30min at room temperature. After serum removal, a primary antibody (anti-YKL-40 antibody) was dropped and kept overnight at 4°C. PBS solution

was rinsed 3 times, and then the biotin-labeled secondary antibody working solution was dropped and incubated at 37°C for 30min. After washing with PBS solution 3 times, the working solution of streptomycin labeled with horseradish enzyme was dropped and incubated at 37°C for 30min. After the above, PBS solution was used for further washing for 3 times, and then DAB was used for color development. Then, tap water was used for washing and hematoxylin was used for staining. After the alcohol was dehydrated to transparent, a neutral adhesive was used for sealing and films were taken under an inverted fluorescence microscope.

The average optical density value was determined (12). Eclipse CI-L photo microscope was used to select the target area of lung tissue for 200-fold imaging. During imaging, tissues should be filled with the whole field as far as possible to ensure the consistency of background light in each photo. After the imaging was completed, image-Pro Plus 6.0 analysis software was used to measure the positive cumulative optical density values of the three fields in each section with the unified pixel area as the standard unit, denoted as A. And the corresponding tissue pixel area was denoted as B, and the average optical DENSITY = A/B was calculated as C.

Western blotting

The expression of β -catenin, e-cadherin and lung surfactant-associated protein C in mouse lung tissue was determined by western blot. The specific steps are to take 10g of lung tissues of the above groups for full grinding, add cell lysis solution containing protease inhibitor in a ratio of 1:9, and then shake it evenly with a low-temperature operating homogenizer. After 30 minutes of full cracking, a pipette was used to move it to the centrifugal tube and centrifuged it at 12000r/min at 4°C for 5 minutes. Then the supernatant was taken and the protein concentration in the supernatant sample was detected by BCA protein concentration, and the protein concentration was adjusted to be consistent. After boiling for 10min, 50ug total protein was taken in sodium dodecyl sulfate-polyacrylamide gel for electrophoresis. The classified protein was transferred to the PVDF membrane by a wet membrane transfer device, and then the membrane was sealed overnight with 5% skim milk powder solution at 4°C. Then, the membrane was washed with TNST buffer solution for 2mi and the primary antibodies, namely β -catenin, e-cadherin and lung surfactant-related protein C protein antibodies, were added respectively, and sealed at room temperature for 2h.

TBST solution was given to clean the membrane 3 times, and the secondary antibodies were added and incubated for 1h without light. Then TBST solution was used 3 times, 10min each time. GAPDH protein was used as the internal reference protein, and the absorbance value of the band was analyzed by the gel imaging system. The relative protein expression is the ratio of the absorbance value of the target band to that of the reference protein.

Determination of mRNA expression by fluorescence quantitative PCR

Total RNA was extracted from the lung tissue of mice with idiopathic pulmonary fibrosis by TRIzol reagent extraction box, and the concentration and purity of total RNA were determined by ultraviolet spectrophotometry. RNA was transcribed into cDNA using a reverse transcription kit. Then real-time fluorescence quantitative PCR reaction was performed using cDNA as an amplification template under PCR reaction amplifiers. Relative quantitative analysis was performed using the $2^{-\Delta\Delta CT}$ method with GAPDH as an internal reference. Specific parameters of the amplifiers were carried out according to the corresponding instructions. The primer sequences are listed in table 1.

Statistical methods

SPSS20.0 statistical software was used for data processing. All data were tested for normal distribution and homogeneity of variance. Measurement data were expressed as standard deviation \pm mean, t-test was used, and analysis of variance was used for inter-group comparison. Enumeration data were expressed as cases or percentages and the chi-square test was used. $P < 0.05$ was considered a statistically significant difference.

Results

Lung wet/dry weight ratio of the four groups

There was no significant difference in body weight among the four groups ($P > 0.05$), and no mice died after lentivirus transfection. In lung wet/dry weight ratio, compared with the CK group, yKL-40-NC group, YKL-40-inhibitor group and YKL-40-MIMics group were significantly increased, the differences were statistically significant ($P < 0.05$), and compared with YKL-40-NC group, The lung wet/dry weight ratio of the YKL-40-inhibitor group was significantly decreased, while that of the YKL-40-MIMics group was significantly increased, with statistical significance ($P < 0.05$), as shown in Table 2.

Table 1. Primer sequences.

Primer	Upstream	Downstream
vimentin	AGATATCTCGCTGCGTCGAA	GATTACGCGTCACGCGTCCGAA
hydroxyproline	GCATTCTCGCGTAAATATAA	GCGCCATCGAATAGCGTATGC
E-cadherin	ATATAGGACCGCGTATGCCA	CTATGCGCAGCGTATATATGA
GAPDH	GAGTCAACGGATTGGTTCGT	TTGATTTTGGAGGGATCTCG

Table 2. Lung wet/dry weight ratio of rats in the four groups.

Group	YKL-40-mimics (n=10)	YKL-40- inhibit (n=10)	YKL-40-NC (n=10)	CK (n=10)	F	P
BMI	22.15 \pm 2.11	22.32 \pm 1.35	22.21 \pm 1.42	22.37 \pm 1.55	5.314	0.012
Wet/Dry	8.92 \pm 0.45 ^{ab}	6.74 \pm 0.21 ^{ab}	7.48 \pm 0.25 ^a	4.71 \pm 0.15	6.523	0.001

Note: Compared with the CK group, a $P < 0.05$ was statistically significant; Compared with the YKL-40-NC group, b $P < 0.05$ was statistically significant.

Lung histopathology of rats in four groups

In the CK group, a small amount of inflammatory cells such as macrophages and neutrophils were found in the lung interstitium, while the alveolar septum was slightly widened, and other abnormalities were not obvious. In the YKL-40-NC group, local alveolar collapse, exudation of a large number of inflammatory cells, and proliferation of fibroblasts and capillaries were observed, and fibrous tissue was found to be reticulated and floc distributed around the trachea and in the interstitium, proving that the mice with idiopathic pulmonary fibrosis were successfully modeled in this study, as shown in Figure 1.

Immunohistochemical analysis and protein expression of YKL-40 in four groups of rats

Compared with the CK group, the AOD value and protein expression level of the YKL-40-NC group, YKL-40-inhibitor group and YKL-40-MIMics group were significantly increased ($P < 0.05$), and compared with the YKL-40-NC group, AOD value and protein expression of YKL-40 were significantly decreased in the YKL-40-inhibitor group, while the AOD value and protein expression of YKL-40 were significantly increased in the YKL-40-MIMics group, with statistical significance ($P < 0.05$), suggesting that lentivirus was successfully transfected, as shown in Figures 2 and 3.

Analysis of proteins related to the interstitial transformation of alveolar epithelial cells in four groups

Compared with the CK group, β -catenin in the yKL-40-NC group was significantly increased, while e-cadherin and Pro-SPC were significantly decreased, the difference was statistically significant ($P < 0.05$). Compared with the YKL-40-NC group, β -catenin N in the YKL-40-NC group was significantly decreased. E-cadherin and Pro-SPC are elevated. β -catenin increased significantly in the YKL-40-MIMics group, while E-cadherin and Pro-SPC decreased significantly. The above differences were statistically significant ($P < 0.05$), as shown in Table 3.

The mRNA expression analysis of pulmonary fibrosis-related factors in the four groups

Compared with CK group, the mRNA expression of vimentin was significantly increased, while the mRNA expression of E-cadherin and hydroxyproline was decreased, with statistical significance ($P < 0.05$). Compared with the YKL-NC group, the mRNA expression of vimentin in the YKL-40-MIMics group was significantly increased, while

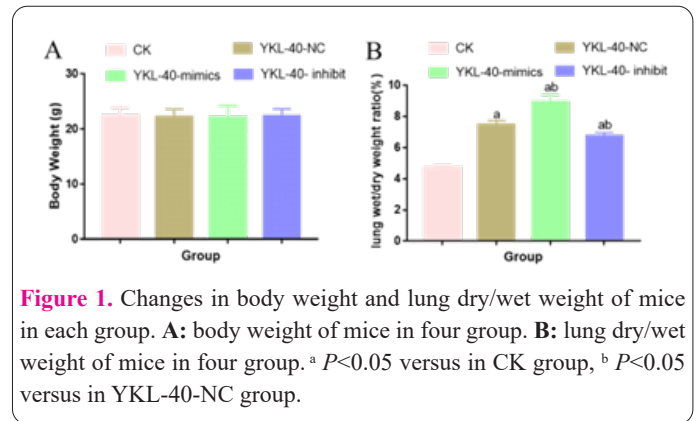


Figure 1. Changes in body weight and lung dry/wet weight of mice in each group. **A:** body weight of mice in four group. **B:** lung dry/wet weight of mice in four group. ^a $P < 0.05$ versus in CK group, ^b $P < 0.05$ versus in YKL-40-NC group.

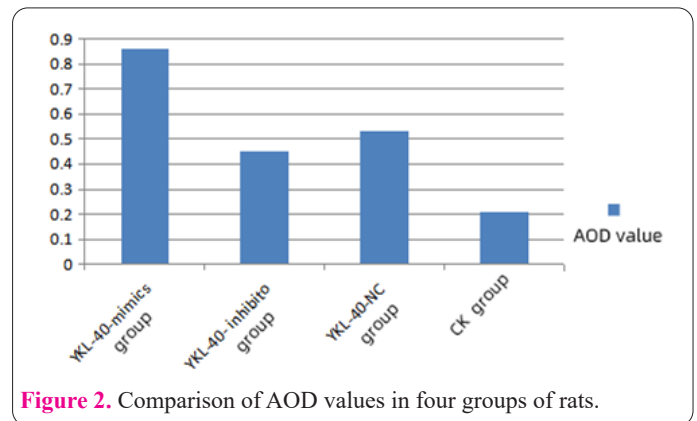


Figure 2. Comparison of AOD values in four groups of rats.

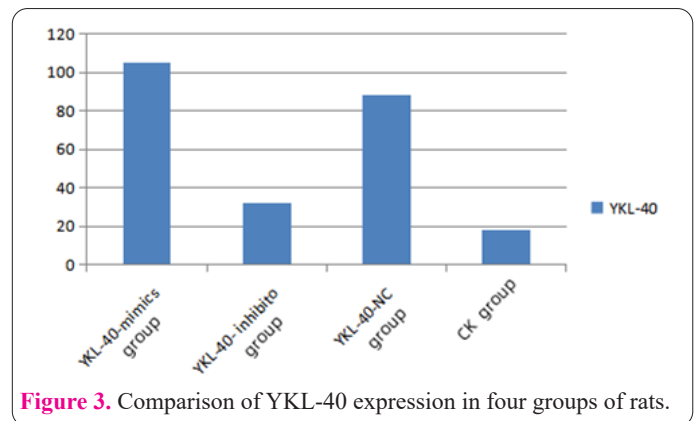


Figure 3. Comparison of YKL-40 expression in four groups of rats.

the mRNA expression of E-cadherin and hydroxyproline was decreased, but the mRNA expression of vimentin in the YKL-40-inhibitor group was significantly decreased. The mRNA expressions of E-cadherin and hydroxyproline were significantly increased, with statistical significance ($P < 0.05$), as shown in Table 4.

Table 3. Analysis of interstitial transformation-related proteins in four groups of alveolar epithelial cells.

Group	YKL-40-mimics (n=10)	YKL-40-inhibitor (n=10)	YKL-40-NC (n=10)	CK (n=10)	F	P
β -catenin	2.05 \pm 0.42 ^{ab}	1.12 \pm 0.19 ^{ab}	1.86 \pm 0.27 ^a	0.95 \pm 0.18	5.378	0.012
E-cadherin	0.92 \pm 0.11 ^{ab}	1.34 \pm 0.57 ^{ab}	1.16 \pm 0.27 ^a	2.05 \pm 0.32	6.541	0.007
Pro-SPC	0.62 \pm 0.11 ^{ab}	0.95 \pm 0.36 ^{ab}	0.84 \pm 0.21 ^a	1.75 \pm 0.12	5.332	0.006

Note: Compared with CK group, a $P < 0.05$ was statistically significant; Compared with the YKL-40-NC group, b $P < 0.05$ was statistically significant.

Table 4. mRNA expression analysis of pulmonary fibrosis-related factors in the four groups.

Group	YKL-40-mimics (n=10)	YKL-40-inhibitor (n=10)	YKL-40-NC (n=10)	CK (n=10)	F	P
vimentin	2.36 \pm 0.42 ^{ab}	1.75 \pm 0.23 ^{ab}	2.14 \pm 0.43 ^a	1.23 \pm 0.32	5.312	0.012
hydroxyproline	0.95 \pm 0.11 ^{ab}	1.32 \pm 0.05 ^{ab}	1.05 \pm 0.21 ^a	1.87 \pm 0.24	6.413	0.003
E-cadherin	0.72 \pm 0.18 ^{ab}	1.59 \pm 0.15 ^{ab}	1.32 \pm 0.15 ^a	1.68 \pm 0.12	5.142	0.007

Note: Compared with CK group, a $P < 0.05$ was statistically significant; Compared with the YKL-40-NC group, b $P < 0.05$ was statistically significant.

Table 5. Expression analysis of TGF- β 1-related pathway proteins in the four groups.

Group	YKL-40-mimics (n=10)	YKL-40- inhibitor (n=10)	YKL-40-NC (n=10)	CK (n=10)	F	P
TGF- β 1	6.15 \pm 0.22 ^{ab}	4.54 \pm 0.12 ^{ab}	5.84 \pm 0.13 ^a	2.23 \pm 0.15	9.812	0.012
Smad3	1.95 \pm 0.11 ^{ab}	1.32 \pm 0.12 ^{ab}	1.69 \pm 0.17 ^a	0.85 \pm 0.07	10.513	0.007
Smad7	2.85 \pm 0.13 ^{ab}	1.23 \pm 0.01 ^{ab}	1.64 \pm 0.14 ^a	0.94 \pm 0.05	6.445	0.009
α -SMA	3.24 \pm 0.41 ^{ab}	1.82 \pm 0.15 ^{ab}	2.57 \pm 0.35 ^a	1.21 \pm 0.23	7.142	0.018

Note: Compared with CK group, aP<0.05 was statistically significant; Compared with the YKL-40-NC group, bP<0.05 was statistically significant.

Expression analysis of TGF- β 1-related pathway proteins in four groups

Compared with the CK group, the expression levels of TGF- β 1, Smad3, Smad7 and α -SMA were significantly increased compared with the CK group, and the differences were statistically significant (P<0.05). Compared with the yKL-NC group, the expression of TGF- β 1, Smad3, Smad7 and α -SMA proteins in the YKL-40-MIMics group were significantly increased. However, TGF- β 1, Smad3, Smad7 and α -SMA protein expressions were significantly decreased in the yKL-40-inhibitor group, and the above differences were statistically significant (P<0.05). Therefore, it is speculated that YKL-40 may promote the interstitial transformation of alveolar epithelial cells through TGF- β 1/Smad3 signaling pathway, as shown in Table 5.

Discussion

Idiopathic pulmonary fibrosis is a chronic, progressive, fibrotic interstitial pneumonia, which is the most common type of idiopathic interstitial pneumonia clinically. The pathological characteristics of this disease are mainly manifested as type II alveolar epithelial hyperplasia, myofibroblast aggregation and pulmonary parenchyma remodeling (13). At present, most studies believe that this disease may be related to the abnormal repair of alveolar epithelial cells after repeated micro-injury, which leads to abnormal activation of alveolar epithelial cells and the generation of various growth factors and chemokines to induce the proliferation of fiber and stromal cells (14, 15). Epithelial interstitial transformation plays an important role in this process. Mesenchymal transformation of epithelial cells refers to the process in which epithelial cells are transformed into mesenchymal cells by cytoskeleton remodeling after losing polarity (16). Current research results suggest that this process can be involved in tissue regeneration, organ fibrosis and tumor invasion and metastasis, and endows cells with a variety of cell biological characteristics such as migration, metastasis and induction into stem cells, prevention of apoptosis and aging, and immunosuppression (17). In recent years, studies have confirmed that TGF- β 1 can induce epithelial interstitial transformation through a variety of cellular pathways and participate in the formation of pulmonary fibrosis (18). In previous studies, YKL-40 was considered as a possible serological marker of granulocyte function in tissue inflammation and was also considered as an indicator to evaluate the severity, progression and spread of disease, as well as a predictor to evaluate the prognosis and therapeutic effect of disease (19). However, Guan et al. (20) showed that YKL-40 regulates the proliferation, apoptosis and migration of human bronchial epithelial cells through TGF- β 1/Smads pathway, but no relevant studies have explored the rela-

tionship between YKL-40 and epithelial cell interstitial transformation. Therefore, this study combined with the above results will make the following discussion.

This study results demonstrated the success of bleomycin in constructing an idiopathic pulmonary fibrosis mouse model, the success of YKL-40 overexpression and lentivirus transfection, the significant change of lung wet/dry weight ratio in mice, and the correlation between YKL-40 and idiopathic pulmonary fibrosis. The lung wet/dry weight ratio to A certain extent represents the size of the degree of damage in patients with pulmonary fibrosis, the higher the ratio value, the more serious the damage, such as Chen et al. (21) study, the results of the study showed in fibrosis of oleic acid in rats model of acute lung injury model of rats lung wet/dry weight ratio higher than normal mice, the tanshinone II - A sulfonic acid sodium decreases after the intervention. Adegunsoye et al. (22) showed that YKL-40 can be used as a prognostic marker for anti-fibrosis therapy in patients with idiopathic pulmonary fibrosis. In this study, compared with the CK group, the yKL-40-NC group, YKL-40-inhibitor group and YKL-40-MIMics group all increased significantly, and compared with the YKL-40-NC group, the lung wet/dry weight ratio of YKL-40-inhibitor group decreased significantly. However, the level of YKL-40-MIMics group was significantly increased. Therefore, combined with the above results, it can be determined that the level of YKL-40 is closely related to the degree of idiopathic pulmonary fibrosis and lung injury in mice.

Also, this study results indicated that there were significant differences in the expression of E-cadherin, Pro-SPC and β -catenin in the yKL-40-NC group, YKL-40-inhibitor group, YKL-40-MIMics group and CK group. The mRNA expressions of e-cadherin, hydroxyproline and vimentin were also significantly different. β -catenin has been confirmed to be abnormally activated in idiopathic fibrosis and participate in epithelial interstitial transformation (23). For example, Okazaki et al. (24) showed that by giving the Wnt/ β -catenin/CBP signal transduction inhibitor PRI-724 to bleomycin-induced pulmonary fibrosis mice, the disease of the mice could be improved by regulating the activity of macrophages in the lung. Pro-spc is the active substance on the alveolar surface, which is mainly synthesized by alveolar epithelial cells and participates in the repair process of alveolar epithelial cells. Therefore, the down-regulation of Pro-SPC can represent the apoptosis of alveolar epithelial cells, and Nureki et al. (25) showed that the mutation of the SP-C gene can lead to the occurrence of spontaneous pulmonary fibrosis. E-cadherin is a calcium-dependent transmembrane protein mediating the adhesion between homogenous cells. It has been previously confirmed that this protein is related to tumor genesis and metastasis, and a large number of studies have

confirmed that a variety of signaling pathways can regulate epithelial-mesenchymal transformation by targeting E-cadherin. In addition, it has been reported that the main characteristics of epithelial interstitial transformation process include decreased expression of E-cadherin and transformation of cytoskeleton of cytokeratin into cytoskeleton dominated by vimentin, while hydroxyproline mainly exists in collagen (26, 27). Due to the characteristics of the above markers, β -catenin and β -catenin in the three groups were significantly increased compared with the CK group in this study, while E-cadherin and Pro-SPC indicated the pathological characteristics of idiopathic pulmonary fibrosis in the three groups. Compared with the YKL-NC group, the mRNA expression of vimentin in the YKL-40-MIMics group was significantly increased, while the mRNA expression of E-cadherin and hydroxyproline was decreased. However, the mRNA expression of vimentin in the YKL-40-inhibitor group was significantly decreased. The expression of E-cadherin and hydroxyproline mRNA was significantly increased, suggesting that the overexpression of YKL-40 promoted the pathological process of alveolar epithelial interstitial transformation, which could be inhibited by YKL-40 knockdown. Therefore, the mechanism of YKL-40 participating in the interstitial transformation of alveolar epithelial cells in mice with idiopathic fibrosis was proved.

Finally, in order to further explore the specific mechanism of YKL-40 promoting the interstitial transformation of alveolar epithelial cells, proteins related to TGF- β 1 pathway were determined in this study. Results 6 showed that compared with CK group, the expression of TGF- β 1, Smad3, Smad7 and α -SMA proteins were significantly increased. The expressions of TGF- β 1, Smad3, Smad7 and α -SMA proteins in the YKL-40-MIMics group were significantly increased, but the expressions of TGF- β 1, Smad3, Smad7 and α -SMA proteins in YKL-40-inhibitor group were significantly decreased. It is suggested that YKL-40 may promote the physiological process of alveolar epithelial cell interstitial transformation through TGF- β 1/Smads signaling pathway. Among them, TGF- β 1/Smad signaling pathway has been proven to be closely related to the occurrence and development of idiopathic pulmonary fibrosis and epithelial interstitial transformation in recent years. For example, Zhou et al. (28) showed that Gly-His-Lys inhibited bleomycin-induced pulmonary fibrosis progression, inflammatory response and interstitial transformation through TGF- β 1/Smad 2/3 and IGF-1 pathways. Li et al. (29) showed that regofinib alleviates bleomycin-induced pulmonary fibrosis by inhibiting TGF- β 1/Smad signaling pathway. In terms of its relationship with YKL-40, Guan et al. 's previous results also showed that YKL-40 regulates the proliferation, apoptosis and migration of human bronchial epithelial cells through TGF- β 1/Smads pathway. Therefore, it is speculated that YKL-40 may promote the physiological process of alveolar epithelial cell interstitial transformation through TGF- β 1/Smads signaling pathway. However, further in vitro cell experiments and targeted luciferase are needed to identify upstream and downstream factors and direct associations.

In conclusion, overexpression of YKL-40 can promote the progression of pulmonary fibrosis and interstitial transformation of alveolar epithelial cells in mice with idiopathic fibrosis, possibly through the TGF- β 1/Smad3 signaling pathway.

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